TABLE I.

	Group				M	
	0	A	B	AB	+	—
Pure Blackfeet and	36	138	0	2	149	3
Blood Indians (this study)	20.5	78.4	0	1.1	98	2
Pure Kansas Indians	156	49	0	0	195	10
(Landsteiner and Levine[7])	76.1	23.9			95.1	4.9
White, Montana	84	59	26	6	130	43
(this study)	48	34	15	3	75	25
White, New York	758	648	226	76	1382	326
(Landsteiner and Levine[7])	44.4	37.9	13.2	4.5	80.9	19.1

The first line of figures indicates absolute numbers examined; the figures below give the corresponding percentages.

Of the "Blackfeet" Indians, 24 were in Group O, 82 in Group A, and 1 in AB; of these only 2 were M negative. Of the "Blood" tribes, 12 were in Group O, 56 in Group A, and 1 in AB. M tests were made on 45 of these specimens and only one was negative.

of difficulty of interpreting certain taste reactions, as for instance, those reported as sweet.

8347

Susceptibility to Lysozyme of Staphylococci.

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In his review on lysozyme Fleming[1] has stressed the fact that the extreme susceptibility of many air saprophytes has resulted in a general impression that pathogenic organisms are not affected by this enzyme. He cites his own and other work to prove that pathogenic organisms are attacked if the concentration of lysozyme is great enough. Ridley[2] reported that the ability to resist lysozyme seemed to determine pathogenicity. Regarding staphylococci, Neisser[3] quotes Nakmura and also Kopp as presenting evidence that susceptibility to lysozyme is to be regarded as indicating that a staph-

[1] Fleming, A., *Proc. Roy. Soc. Med.*, 1932, **26**, 71.

[2] Ridley, F., *Proc. Roy. Soc. Med.*, 1928, **21**, 1495.

[3] Neisser, M., *Handbuch der path. Microorg.*, Kolle, Kraus and Uhlenuth, Berlin, 1927, **4**, 437.

ylococcus is not a "Pyococcus", *i. e.*, of the pathogenic type. Cavka and Prica[4] found that tears acted only upon air staphylococci and not upon those isolated from lesions.

In experiments with a number of strains of staphylococci and sarcinae, we have observed a certain relationship between type of pigment, mannite fermentation and coagulase production on the one hand and susceptibility to lysis by lysozyme on the other, which we believe justifies a brief report. Since the work of Gordon[5] and Hine[6] it has been generally accepted that mannite-fermenting staphylococci are more likely to be pathogenic than those which do not have this property. Much,[7] Gross[8] and others have shown that pathogenic staphylococci have the power of coagulating oxalated human plasma. This action has been ascribed to an enzyme termed coagulase. The study of the cultures reported here has been limited to the above mentioned properties. Hemolysin, skin toxin, leucocidin or the production of specific antigen have not been studied.

Lysozyme Test. Saline suspensions of 18-24-hour agar-slant cultures of the various organisms were made to produce a turbidity equivalent to a No. 8 barium sulphate standard. 0.5 cc. amounts of progressive saline dilutions of fresh egg-white or of purified,* concentrated lysozyme from egg-white were added to equal quantities of the bacterial suspensions in small test tubes. The tubes were incubated in a water bath at 37°C. for 24 hours and readings made by comparing the turbidities with that of a control tube of organisms plus saline. The efficacy of the lysozyme preparation was controlled by titration with a sarcina of known susceptibility.

Mannite Fermentation. Cultures were inoculated into tubes of 1% Difco mannite in beef infusion broth. After 3 days' incubation the presence of acid was tested for by adding 2 drops of bromcresol purple.

Coagulase. Oxalated plasma was obtained by placing 10 cc. of fresh human blood into a centrifuge tube containing 0.02 gm. of potassium oxalate, thoroughly mixing and then centrifuging out the cells. 0.3 cc. of the plasma was mixed with 0.2 cc. of an 18-24-hour

4 Cavka, V., and Prica, M., *Arch. f. Ophth.*, 1929, **121**, 740.

5 Gordon, M. H., Rep. (34th) Med. Off. Local Govt. Bd., 1906, 387.

6 Hine, T. G. M., *Lancet*, 1922, **2**, 1380.

7 Much, H., *Biochem. Z.*, 1908, **14**, 143.

8 Gross, H., *Cent. Bact. I Orig.*, 1931, **122**, 354.

*Methods of purification described elsewhere.[9]

9 Meyer, K., Palmer, J. W., Thompson, R., and Khorazo, D., *J. Biol. Chem.*, in press.

broth culture of the organism being tested. Partial or complete clotting after 2 hours' incubation at 37°C. indicated the production of coagulase.

Pigment. The pigment was classified as orange, lemon or white by the appearance of the massed growth from a 72-hour agar plate placed upon white filter paper.

One hundred and twenty strains of staphylococci and sarcinae from various sources, chiefly normal and inflamed conjunctivae were tested by these methods. The results are summarized in Table I.

TABLE I.

Classified by	Total No. of strains	No. of strains dissolved by lysozyme	% of strains dissolved by lysozyme
	Staphylococci		
Pigment			
Orange	60	10	16
White	39	19	48
Lemon	4	4	100
Mannite Fermentation			
Positive	54	4	8
Negative	49	29	59
Coagulase Production			
Positive	64	7	10
Negative	39	22	56
	Sarcinæ		
Pigment			
White	5	5	100
Lemon	11	11	
Rose	1	1	

None of the sarcinae or the lemon staphylococci fermented mannite or produced coagulase. Seventy-eight percent of the orange strains and 18% of the white strains fermented mannite. Eighty-three percent of the orange strains and 36% of the white strains produced coagulase.

For simplification the various degrees of susceptibility to lysozyme have not been shown. The lemon staphylococci and all the sarcinae showed a sensitivity to lysozyme approaching or as great as that of the test organism. The white and orange staphylococci in no case showed marked susceptibility. The suspensions of these were seldom completely dissolved and in most cases only partial clearing occurred in a lysozyme dilution of 1-4 or 1-8.

Summary. All strains of sarcinae (17) and lemon staphylococci (4) studied were readily dissolved by lysozyme. None of the 99 white or orange staphylococci studied showed marked susceptibility, but certain strains were dissolved by higher concentrations of enzyme. There was a definite, although not absolute, negative corre-

lation between orange pigment, the ability to ferment mannite or produce coagulase on the one hand and susceptibility to lysozyme on the other.

8348 P

Quantitative Use of Neufeld Reaction with Special Reference to Titration of Type II Antipneumococcic Horse Sera.

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The Neufeld reaction has recently been advocated[1] for the diagnosis of the different types of pneumonia directly from the patient's sputum. For this purpose, he advised a monovalent antipneumococcic rabbit serum in order to avoid the non-specific reactions which he obtained with the use of antipneumococcic horse serum. More recently, Cooper and Walter[2] reported this same "swelling" phenomenon when antipneumococcic serum from rabbits was mixed with the homologous pure culture grown in artificial media. This observation led us to examine different samples of antipneumococcic serum by mixing them with specific pure cultures and observing the degree of capsular swelling which resulted. It was found that a serum high in mouse protective units caused much more swelling than a serum low in mouse protective units, also that a serum of high potency could be diluted many times and still give a typical "swelling" reaction. These experiments suggested a possible quantitative application of the Neufeld reaction.

Preliminary work with Type II broth cultures and specific antipneumococcus horse sera showed a definite linear relationship between the number of organisms used and the least amount of antibody required to produce an enlarged capsule. This reaction appeared to be specific. The following method of titration of Type II antibody was then developed:

The culture, either live or formalinized, is diluted with 1% peptone to the required density; the serum is diluted with physiological saline solution. Appropriate amounts of a constant culture dilution and of varying serum dilutions are measured with standard platinum loops, placed on a thin cover-slip, mixed well with a loopful of

[1] Sabin, *J. A. M. A.*, 1933, **100**, 1584.

[2] Cooper and Walter, *Am. J. Pub. Health*, 1935, **25**, 469.